Pregnancy at high altitude in the Andes leads to increased total vessel density in healthy newborns

Gassmann, Norina N ; van Elteren, Hugo A ; Goos, Tom G ; Morales, Claudia R ; Rivera-Ch, Maria ; Martin, Daniel Stuart ; Cabala Peralta, Patricia ; Passano Del Carpio, Augustin ; Aranibar Machaca, Saul ; Huicho, Luis ; Reiss, Irwin K M ; Gassmann, Max ; de Jonge, Rogier C J

Abstract: The developing human fetus is able to cope with the physiological reduction in oxygen supply occurring in utero. However, it is not known if microvascularisation of the fetus is augmented when pregnancy occurs at high altitude. Fifty-three healthy term newborns in Puno, Peru (3,840m) were compared to sea level controls. Pre- and post-ductal arterial oxygen saturation (SpO2) was determined. Cerebral and calf muscle regional tissue oxygenation were measured using near infrared spectroscopy (NIRS). Skin microcirculation was non-invasively measured using Incident Dark Field imaging. Pre- and post-ductal SpO2 in Peruvian babies was 88.1% and 88.4% respectively, which was 10.4% and 9.7% lower than in newborns at sea level (p<0.001). Cerebral and regional oxygen saturation were significantly lower in the Peruvian newborns (cerebral 71.0 % vs. 74.9%; regional 68.5% vs. 76.0%, p<0.001). Transcutaneously measured total vessel density in the Peruvian newborns was 14% higher than that in the newborns born at sea level (29.7 vs. 26.0 mm/mm2; p 0.001). This study demonstrates that microvascular vessel density in neonates born to mothers living at high altitude is higher than that in neonates born at sea level.

DOI: https://doi.org/10.1152/japplphysiol.00561.2016

Articles in PresS. J Appl Physiol (July 21, 2016). doi:10.1152/japplphysiol.00561.2016

Pregnancy at high altitude in the Andes leads to increased total vessel density in healthy newborns

Norina N. Gassmann[1,2+], Hugo A. van Elteren[2+], Tom G. Goos[2,3], Claudia R. Morales[4], Maria Rivera[4,5], Daniel S. Martin[6], Patricia Cabala Peralta[7], Agustin Passano del Carpio[7], Saul Aranibar Machaca[7], Luis Huicho[5,8], Irwin K.M. Reiss[2], Max Gassmann[1,8§*], Rogier C.J. de Jonge[2§]

[1]Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology (ZIHP), Medical Faculty, University of Zurich, Switzerland; [2]Department of Pediatrics, Division of Neonatology, Erasmus MC – Sophia Children's Hospital, University Medical Center, Rotterdam, The Netherlands, [3]Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands, [4]Laboratory of Adaptation to High Altitude, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru, [5]Center of Research for Integral and Sustainable Development (CIDIS), UPCH, Lima, Peru, [6]University College London Centre for Altitude Space and Extreme Environment Medicine, UCLH NIHR Biomedical Research Centre, Institute of Sport and Exercise Health, London, United Kingdom, [7]Hospital III Puno EsSalud, Puno, Peru, [8]School of Medicine, UPCH, Lima, Peru.

[+]These authors contributed equally to this work

[§]The contribution of both senior authors was equivalent

*Corresponding author: maxg@access.uzh.ch

Abstract

The developing human fetus is able to cope with the physiological reduction in oxygen supply occurring *in utero*. However, it is not known if microvascularisation of the fetus is augmented when pregnancy occurs at high altitude. Fifty-three healthy term newborns in Puno, Peru (3,840m) were compared to sea level controls. Pre- and post-ductal arterial oxygen saturation (SpO_2) was determined. Cerebral and calf muscle regional tissue oxygenation were measured using near infrared spectroscopy (NIRS). Skin microcirculation was non-invasively measured using Incident Dark Field imaging.

Pre- and post-ductal SpO_2 in Peruvian babies was 88.1% and 88.4% respectively, which was 10.4% and 9.7% lower than in newborns at sea level ($p<0.001$). Cerebral and regional oxygen saturation were significantly lower in the Peruvian newborns (cerebral 71.0 % vs. 74.9%; regional 68.5% vs. 76.0%, $p<0.001$). Transcutaneously measured total vessel density in the Peruvian newborns was 14% higher than that in the newborns born at sea level (29.7 vs. 26.0 mm/mm^2; $p\leq 0.001$). This study demonstrates that microvascular vessel density in neonates born to mothers living at high altitude is higher than that in neonates born at sea level.

News and Noteworthy:

The natural hypoxic environment at high altitude results in reduced oxygenation, especially in the growing human fetus. Our prospective observational study on healthy term newborns in Peru (Puno at 3840m) that included novel non-invasive visualization of microcirculation demonstrates that vessel density is elevated by 14% in neonates born to women living at high altitude as compared to babies born at sea level, most likely revealing an early adaptive mechanism to a highly hypoxic antenatal environment.

Key words:

Microcirculation, Oxygen Profiling, Incident Dark Field Imaging, Near Infrared Spectroscopy, Neonates, Hypoxia, Vascularization

Introduction

It is estimated that in the South American Andes over 30 million people - most of them belonging to the Quechua or Aymara population and termed here "Andean" - permanently live above 2,500m (8,200ft), defined as high altitude (2, 10). At high altitude, the environmental conditions are extreme, including dramatic temperature changes and low atmospheric pressure leading to hypobaric hypoxia. The consequences of this are often exacerbated by low socio-economic status and negatively impact upon the health of infants (46). Of note, people living at high altitude not only show genetic adaptation but also plasticity in development in response to hypoxia (1, 17). Despite the harsh conditions at the high altitudes of the Andes, most fetuses develop well and are delivered at term (31). For that matter, it must be understood that the intrauterine environment already represents an extreme surrounding at sea level that is exacerbated in pregnancies at high altitude. In general, proper *in utero* development requires adequate oxygen delivery to the fetus, which is achieved by increased maternal ventilation rate and thus increased blood oxygen saturation (SpO_2) level (22, 25). Under conditions of chronic hypoxia, however, the utero-placental blood flow is lower (16) and, consequently, oxygen uptake by the fetus is reduced. This process can even be exacerbated by the presence of maternal preeclampsia (12). When pregnancy occurs at 3,100m, however, the placenta increases antioxidant capacity (38) while the fetus is able to adapt to maternal and placental hypoxemia by increasing nitric oxide production *in utero* and after birth. This adaptive response might be necessary to sustain placental blood flow but may also lead to improvement of microcirculatory blood flow (28).

It was shown, decades ago, that babies born to indigenous Andean women have a higher birth weight than non-Andean neonates both born at high altitude (9). A more recent study revealed

that elevated uterine artery blood flow and thus increased oxygen delivery protect Andeans from fetal growth retardation when pregnancy occurs at high altitude (16). Perinatal Doppler and ultrasound studies in Andean fetuses performed at 3,600m showed reduced umbilical blood flow, compensated for, however, by the fetuses' elevated neonatal hemoglobin concentration and increased oxygen extraction capability (31). As a result, fetal oxygen delivery and oxygen consumption at high altitude do not differ from values measured at low altitude (31), supporting the notion that the fetus copes with the extreme *in utero* situation by increasing systemic blood flow and thus oxygen delivery. Note that the present study does not include the Tibetan population which is known to maintain better neonatal oxygenation than Andeans (reviewed in (24)).

Apart from vasodilation, an obvious strategy to increase blood and thus oxygen supply to the tissue is to increase microvascular density. Microcirculation studies in critically ill neonates (40) found a low microvascular density to be a predictor for mortality in sepsis (39). However, no studies have reported on the effect of antenatal hypobaric hypoxia on fetal microcirculatory development. Thus, in the present prospective observational study the aim was to obtain microcirculatory profiles of term babies born at high altitude and compare these with the profiles of babies born at sea level. We postulated that the microvascularisation of the neonate born to mothers at high altitude is elevated and that this phenomenon reflects a general adaptive mechanism.

Materials and Methods

Subjects

This prospective observational study was performed in August 2014 at the pediatric department of the Hospital EsSalud III in Puno (Peru) located at 3,840m above sea level. The Peruvian microcirculatory measurements were compared to those performed at sea level in the maternity ward of the Erasmus MC - Sophia Children's Hospital in Rotterdam, the Netherlands (altitude: 0m) where measurements were performed by the same operator using identical instrumentation. Before any measurements were taken, all parents gave their written informed consent. The study protocol was approved by the Ethics Committee of the Universidad Peruana Cayetano Heredia (UPCH 180-17-14; 62794) as well as by the local Ethics Authorities represented by the Red Asistencial Puno EsSalud and the Erasmus MC Rotterdam Ethics Committee (NL48445.078.14). The measurements were carried out in accordance with the approved guidelines. Eligible for participation were healthy, singleton newborns of women either residing at high altitude (Puno and surroundings) or at sea level (Rotterdam and surroundings) at least during pregnancy, delivered either vaginally or by caesarean section, with Apgar scores of 8 or higher and not older than 30 hours at the time of measurement. Newborns were considered healthy if born at term to apparently healthy mothers not suffering from obvious pregnancy complications (no ante- or postnatal abnormalities). Maternal data on smoking was not collected. Babies delivered by caesarean section at high altitude (n=19), but not those at sea level, were placed in an incubator (33°C, 21% O_2) until the mother recovered. The latter babies were measured at a mean of 17h after birth (similar to the vaginal-delivered ones: 14h) and 30 minutes after being taken out of the incubator. The room temperature at which the babies were measured was 22-23°C. Exclusion

criteria included gestational age below 37 or above 42 weeks, any known congenital, hematologic or cardiorespiratory disorder and refusal of written parental informed consent.

We intended to assign ancestry by analyzing the babies' parental surnames, a method that was validated by analyzing ancestry informative genetic markers (4, 45). Babies born to Andean parents acquire both parental surnames that are not changed upon marriage. Accordingly, this custom yields four parental surnames for every child. By the method taking into account this tradition (16, 30), we considered a baby as "indigenous" if she or he had three or four Andean parental surnames. Babies with two Andean and two Hispanic surnames were considered of "mixed origin". If three or four parental surnames were of Hispanic origin, the baby was considered as "Hispanic". Classification was not possible in all other cases. Note that this classification is an approximation only as early reports show that it is not fully accurate to predict non-Andean ancestry using Hispanic surnames (34, 45). Accordingly, the "Hispanic" population cannot be classified as being of low-altitude but as of combined ancestry.

Data collection

Clinical data from 53 healthy term-born neonates born at high altitude, most of them born to Aymara parents, were retrieved from the medical files of the Hospital Puno EsSalud III and clinical data from 33 healthy term-born neonates born at sea level from the medical files of the Erasmus MC - Sophia Children's Hospital. Data included gender, gestational age, birth weight, mode of delivery, and rectal temperature. Additional data - only available in Peruvian newborns - included heart rate, respiratory rate, hematocrit, hemoglobin concentration as well as platelets

and leukocyte count. For assessment of ancestry the surnames of the babies, the mothers and of the fathers (in 19 cases we obtained only one paternal surname instead of two) were collected. Full microcirculatory profiles were obtained by the following measurements performed simultaneously: pre- and post-ductal arterial oxygen saturation (SpO_2), regional and cerebral tissue oxygen (rSO_2 and $crSO_2$) and total vessel density (TVD) using transcutaneous microcirculatory imaging. All newborns were asleep or awake but remain calm during measurements. While full microcirculatory profiles were obtained in Puno, in 33 newborns from Rotterdam only the transcutaneous microcirculation profiles were obtained.

Measurement methods

Pre- and post-ductal arterial oxygen saturation (SpO_2) levels were measured on the right and left wrist using two MASIMO RADICAL 7 pulse oximeters (Masimo Corp., Irvine, CA, USA). Regional tissue oxygen saturation was measured by near infrared spectroscopy (NIRS) using the INVOS device (Somanetics Corp., Troy, Michigan). This device uses near-infrared light at wavelengths of 730 and 810nm to measure oxygenated and deoxygenated hemoglobin. Tissue oxygen saturation, defined as the percentage of oxygenated hemoglobin/total hemoglobin, was measured on the forehead to determine the cerebral oxygen saturation ($crSO_2$) and on the skeletal calf muscle to determine the regional oxygen saturation (rSO_2). Fractional tissue oxygen extraction (FTOE) was calculated as (pre-ductal arterial saturation -cerebral saturation) / pre-ductal arterial saturation [(SO_2 - $crSO_2$)/ SO_2] for cerebral (crFTOE) and with the rSO_2 for the skeletal calf muscle measurements (rFTOE). Pulse oximetry and NIRS measurements of Peruvian newborns were compared to published reference values (13, 27, 29, 41).

Skin microcirculation was measured on the upper inner arm using incident dark field (IDF) technology (Braedius, Huizen, the Netherlands). This device (CYTOCAM) is a handheld microscope with an illumination unit (green light, 450nm) that allows optimal absorption of deoxy- and oxyhemoglobin thereby permitting visualization of the erythrocytes (44). The transcutaneous approach was chosen because sublingual measurement in newborns is not possible and a newborn's skin is thin enough to allow this (43). Identical instrumentation was used in Puno and Rotterdam and the measurements were performed by one and the same technical study operator present at both sites. A minimum of three video clips were recorded and those that did not met the quality criteria according to Massey *et al.* (21) were excluded from further analysis. TVD was automatically analyzed using CCTools (Version 1.7.12, brightness 500, sensibility level 95%). A distinction was made into small vessels, medium and large vessels: Ø ≤10, 10-20 and 20-100 μm, respectively. The automated analysis standardizes the process of analysis and thereby excludes inter-observer variability (42). Following standard guidelines, a minimum of three video clips per newborn was used for automated analysis (5). The microvascular flow index (MFI) and the heterogeneity index (HI) semi-quantitatively describe the velocity of microcirculatory perfusion (5). Each video image was divided in four equally sized quadrants. Each quadrant was scored manually by one experienced operator according to the predominant type of flow (continuous: 3, sluggish [e.g. continuous but very slow]: 2, intermittent: 1, or absent: 0). The MFI is represented by the mean score of the type of flow, and HI by the difference between the highest quadrant and the lowest quadrant score divided by the mean score of all quadrants for one measurement. The MFI and HI for small (Ø ≤10 μm) and non-small vessels (Ø 10 - 100 μm) were determined. This method shows good intra-rater variability and is described in more detail elsewhere (3).

Statistical analysis

Continuous data are presented as median and range for non-normally distributed variables and as mean and standard deviation (SD) for normally distributed parameters. Non-continuous variables are presented as percentages of total and 95% confidence intervals (CI) of proportions. Normally distributed continuous data were compared using an unpaired t-test. Pre- and post-ductal arterial saturation and cerebral saturation were compared with the aforementioned international reference values using a one-sample t-test. Median values were compared using a one sample Wilcoxon signed rank test. One way-ANOVA was used to compare means between more than two groups. Multivariable linear regression analyses adjusting for possible confounding variables were performed using SPSS version 21(IBM Co., Armonk, NY, USA). The crude association between skin microcirculation parameters and country (Peru/Netherlands), was adjusted for sex, gestational age, birth weight z-score, Apgar score (5 minutes), mode of delivery, pregnancy (primigravida/multigravida) and rectal temperature. Collinearity analysis to explore correlation between all covariates using a correlation matrix was performed. A cut-off value of 0.7 was used for the exclusion of variables in the model. Residual plots were constructed to check for normality of the distribution of the residuals.

Results

Comparison of demographic data is shown in Table 1. Gender distribution was approximately even, gestational age and birth weight were similar between Peru and Rotterdam. About one third of the Peruvian newborns were delivered by caesarean section, versus circa 60% in Rotterdam. In Puno, 18 babies were classified as “indigenous”, 6 as “mixed” and 19 as “Hispanic”. The remaining 10 babies could not be classified by surnames. The birth weight of indigenous, mixed and Hispanic newborns was 3,374 (SD 315), 3,325 (SD 414) and 3,196 (SD 220) grams, respectively. Comparison of birth weight between these groups, adjusted for sex and gestational age, showed no significant difference (the comparison between indigenous vs. Hispanic resulting in $p=0.1$). Nevertheless, this trend of higher birth weight in indigenous newborns was in accordance to recent studies (8, 15) reporting that high altitude generally decreases birth weight but that birth weight of neonates of Andean descent was higher than that of neonates of combined origin.

Additional clinical data from the 53 healthy Peruvian newborns (3,840m above sea level) were the following: mean heart rate 145 (SD 13) n/min, mean respiratory rate 53 (SD 5) n/min, mean hematocrit 0.57 (SD 0.06), mean hemoglobin 19.0 (SD 1.9) g/dL, mean platelets count 247 (SD 53 x 10^{-9}) dL and mean leukocytes count 18.6 (SD 4.1 x 10^{-9} dL).

Mean pre- and post-ductal saturation in Peruvian newborns was 88.1% (SD 4.1%) and 88.4% (SD 4.6%), respectively (Fig.1). These values were significantly lower ($p<0.001$) than reference values (13) obtained from a total of 13,714 term newborns at sea-level, that are 98.5 and 98.7%, respectively. The relative difference between pre- and post-ductal saturation in high and low altitude born babies thus was 10.4% and 9.7%, respectively. The results of cerebral and regional

NIRS measurements at high altitude are also shown in figure 1. These data were compared to published reference values of term infants (cerebral n=339 and regional n=72), born at sea level and measured with the same NIRS device (27, 29, 41). Tissue oxygen saturation was significantly lower (cerebral 71.0% vs. 74.9%; calf muscle 68.5% vs. 76.0%, $p<0.001$). Lower arterial and tissue saturation was not associated, however, with different tissue oxygen extraction (crFTOE 0.19 vs. 0.19, p=0.610; rFTOE 0.22 vs. 0.24, p=0.199).

Regarding cutaneous microcirculation data, in only two cases (one from Puno and one from Rotterdam) microcirculation data could not be analyzed due to low quality video imaging and thus both were excluded from further analysis. As for the remaining cases, the mean TVD in the Peruvian babies born was 14% higher than that in the Rotterdam babies (Fig. 2, upper right). Automated morphometric analysis revealed that both, small and medium sized vessels (but not large ones) were significantly longer in the Peruvian newborns (Fig. 2, lower part). To assess as whether ancestry might have an impact on increased microvascularisation in newborns at high altitude, TVD was calculated for the three groups mentioned above: indigenous, mixed and Hispanic (n = 18, 6 and 19, respectively). No statistical differences in TVD were found between any two groups tested. Moreover, there was a remarkable difference in incidence of caesarian sections between the Rotterdam and Puno group (60.6 vs. 35.9%) but we observed no differences in TVD between the two delivery modes (Rotterdam: caesarian section vs. vaginal delivery: mean TVD 25.86 and 26,18 mm/mm^2, respectively, p=0.761; Puno: caesarian section vs. vaginal delivery: mean TVD 29.55 and 29.72 mm/mm^2, respectively, p=0.728).

Multivariable linear regression analysis adjusted for possible confounding variables between countries showed no collinearity between the independent variables used in the model and normal distribution of the residuals. Table 2 shows the corresponding crude and adjusted

differences for microcirculatory parameters: after adjustment the difference between the Peruvian and Rotterdam groups remained significant. Moreover, both the MFI and HI were not altered in either group.

Discussion

Reduced oxygenation of the placenta is linked to severe complications including intra-uterine growth retardation and preeclampsia (12, 23, 36). Of note, despite reductions in systemic oxygen supply, such as occurs at high altitude, the fetus is able to cope with this extreme but still physiologic hypoxic condition. While many studies have addressed the hypoxic placenta's vascular remodeling and metabolic changes (reviewed in (19, 36)), data on the mature fetus's adaptation to a hypoxic environment are scarce. The present study is the first, to our knowledge, to examine microvascular density in healthy term neonates born to mothers that were living at high altitude during pregnancy (3,840m). Our major finding was that their TVD was approximately 14% higher than in neonates born at sea level, pointing towards a possible adaptive fetal strategy to cope with reduced oxygenation. In addition, based on our surname assessment, we suspect that the increase in TVD was independent of the babies' ancestry.

The microcirculation is defined as vessels equal to or smaller than 100µm in diameter that form the capillary network (11). The above-mentioned difference in TVD was still significant when the crude data were adjusted for the following predefined, potentially confounding variables: country, gender, gestational age, birth weight, Apgar score (5min), mode of delivery, primigravida/multigravida, and rectal temperature. Increased vascularization was observed in small (Ø ≤10µm) and medium (Ø 10-20µm) vessels but not in larger ones. This implies that vessel density is only increased at the level of gas exchange (i.e. capillaries and small arterioles).

In a study of healthy adults with no high altitude ancestry (20) a 10.9% increase in TVD was found in subjects first measured at sea level and thereafter at high altitude (5,300m). Also, in preterm infants born small for gestational age, most often caused by more extreme hypoxic conditions, TVD was significantly higher soon after birth (van Elteren *et al.*, unpublished observations).

Considering that blood flow in the umbilical vein is reduced at high altitude (31) and that vascularization seems to be independent of ancestry, it is plausible to speculate that enhanced microvascularisation is a general adaptive mechanism that might be induced by hypoxia-driven stabilization of the α-subunits of the hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) (reviewed in (7, 37)). In turn these heterodimeric regulatory transcription factors up regulate hypoxia-dependent genes including those that trigger angiogenesis such as the vascular endothelial growth factor (VEGF) (26, 36). In contrast to the Andean population, evolution has selected a blunted erythropoietic response for Tibetans as an adaptive strategy to high altitude: a missense mutation in the EGLN1 gene that encodes for the main cellular oxygen sensor results in increased HIFα degradation under hypoxic conditions (18) but this observation has been challenged recently (33). Nevertheless, it would be of interest to determine TVD in healthy babies born to Tibetan mothers at high altitude. Apart from such mutations also epigenetic modifications may support adaptation to exogenous factors such as hypoxia, which can be transmitted to next generations. As such, Julian *et al.* recently provided evidence that unique DNA methylation patterns occur in genes known to influence vascular development and integrity in offspring of hypertensive pregnancies (14).

While the babies' heart rate at high altitude (mean 145, SD 13 n/min) did not deviate from published data, levels of hematocrit (0.57 vs. 0.49-0.50) and hemoglobin concentration (19.0 vs. 16.8 -17.1 g/dl) values in our Peruvian population were higher than those reported in a study performed at 3,600m (31). We cannot explain this difference as the hospital in which our study was conducted was located only about 300m higher. Nevertheless, in the present study the flow-related parameters MFI and HI did not differ between the high-altitude and sea level groups despite a physiological higher hematocrit level in the high-altitude group. However, hematocrit values measured in arterial or venous blood differ greatly from hematocrit at a microcirculatory level. Known as tube hematocrit, it is significantly lower and highly variable in the presence of a constant systemic hematocrit (6). Systemic hematocrit is therefore not correlated to viscosity and blood flow at a microcirculatory level. Moreover, it should be noted that MFI values are often lower in disease states, especially in individuals suffering from septic shock (32).

Previously, a study on NIRS measurements in 24 children reported a significant decrease in cerebral tissue oxygen saturation on ascent from 1610m to 3109m (78% to 67%, $p<0.001$) (47). In another study, reporting NIRS measurement in 17 children during emergency helicopter transport, NIRS decreased from 69.2% to 66.3% in patients transported to altitudes higher than 5000ft (1524m) above sea level (35). Although these two studies measured the response to acute hypoxia, these observations are in line with our results showing that exposure to high altitude significantly lowers cerebral tissue oxygenation.

Limitations

Due to unforeseen administrative delays in Peru, measurements could not be performed in the local sea level control group that of note is mainly represented by a Hispanic population. Therefore, measurements at high altitude were compared to sea level values either found in the literature (pulse oximeter and NIRS data) or by own data obtained from our Rotterdam cohort (determination of TVD). Although a control group of babies born at sea level in Peru is also not completely similar to the neonates in Puno, the use of a Dutch control group might have introduced additional unknown confounding factors. The number of participants in the referred studies exceeded the number of participants in our control group, thereby serving as a reliable comparison group unless ancestry plays an important role. This was assessed and despite the fact that all four parental surnames of the neonates were not always obtained, it was possible to classify a significant number as indigenous (n=18) or Hispanic (n=19). Although ancestry classification by surname is not as precise as genetic analysis, this strategy - first being described and validated back in 1989 (4) - has been successfully applied recently (30, 34). Considering that elevated TVD was observed in all analyzed neonates who consisted of Andean and combined ancestry, we propose that comparison of our data obtained in neonates born at high altitude to sea level neonates from the literature is sound.

The automated computer IDF technology used for microcirculatory analysis has, just like its predecessor methods (sidestream darkfield imaging and orthogonal polarization spectral imaging), only been validated against its predecessor. However, given that the same method was used in both the Peruvian and the Rotterdam group, under supervision of the same experienced operator, any limitation of the software should be equally reflected in both groups. Thus, the data provided are comparable within this study but cannot be extrapolated to other studies.

To conclude, in this study, microvascular vessel density measured using IDF imaging was higher in babies born at high altitude than in babies born at sea level. Neonatologists are often confronted with hypoxemia in infants due to cardiorespiratory insufficiency and prematurity. Visualizing the cutaneous microcirculation represents a new, non-invasive and fast diagnostic tool in neonatal intensive care helping to understand the balance between macrocirculation and peripheral perfusion and tissue oxygenation in newborns.

References

1. **Beall CM**. Human adaptability studies at high altitude: research designs and major concepts during fifty years of discovery. *Am J Hum Biol* 25: 141-147, 2013.
2. **Beall CM**. In: *High Altitude: Human Adaptation to Hypoxia*. New York: Springer Science + Business Media, 2014, p. 357-377
3. **Boerma EC, Mathura KR, van der Voort PH, Spronk PE, and Ince C**. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care* 9: R601-606, 2005.
4. **Chakraborty R, Barton SA, Ferrell RE, and Schull WJ**. Ethnicity determination by names among the Aymara of Chile and Bolivia. *Hum Biol* 61: 159-177, 1989.
5. **De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, and Ince C**. How to evaluate the microcirculation: report of a round table conference. *Crit Care* 11: R101, 2007.
6. **Desjardins C, and Duling BR**. Microvessel hematocrit: measurement and implications for capillary oxygen transport. *Am J Physiol* 252: H494-503, 1987.
7. **Franke K, Gassmann M, and Wielockx B**. Erythrocytosis: the HIF pathway in control. *Blood* 122: 1122-1128, 2013.
8. **Giussani DA, Phillips PS, Anstee S, and Barker DJ**. Effects of altitude versus economic status on birth weight and body shape at birth. *Pediatr Res* 49: 490-494, 2001.
9. **Hass JD, Frongillo EA, Jr., Stepick CD, Beard JL, and Hurtado L**. Altitude, ethnic and sex differences in birth weight and length in Bolivia. *Hum Biol* 52: 459-477, 1980.
10. **Hornbein T, Schoene, R.** In: *Lung Biology in Health and Disease.* Tayler & Francis, 2001.
11. **Ince C**. The microcirculation is the motor of sepsis. *Crit Care* 9 Suppl 4: S13-19, 2005.
12. **Jayet PY, Rimoldi SF, Stuber T, Salmon CS, Hutter D, Rexhaj E, Thalmann S, Schwab M, Turini P, Sartori-Cucchia C, Nicod P, Villena M, Allemann Y, Scherrer U, and Sartori C**. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. *Circulation* 122: 488-494, 2010.
13. **Jegatheesan P, Song D, Angell C, Devarajan K, and Govindaswami B**. Oxygen saturation nomogram in newborns screened for critical congenital heart disease. *Pediatrics* 131: e1803-1810, 2013.
14. **Julian CG, Pedersen BS, Salmon CS, Yang IV, Gonzales M, Vargas E, Moore LG, and Schwartz DA**. Unique DNA Methylation Patterns in Offspring of Hypertensive Pregnancy. *Clin Transl Sci* 8: 740-745, 2015.
15. **Julian CG, Vargas E, Armaza JF, Wilson MJ, Niermeyer S, and Moore LG**. High-altitude ancestry protects against hypoxia-associated reductions in fetal growth. *Arch Dis Child Fetal Neonatal Ed* 92: F372-377, 2007.
16. **Julian CG, Wilson MJ, Lopez M, Yamashiro H, Tellez W, Rodriguez A, Bigham AW, Shriver MD, Rodriguez C, Vargas E, and Moore LG**. Augmented uterine artery blood flow and oxygen delivery protect Andeans from altitude-associated reductions in fetal growth. *Am J Physiol Regul Integr Comp Physiol* 296: 1564-1575, 2009.
17. **Little MA, Baker, P. T.** In: *Environmental Adaptations and Perspectives.* International Biological Program. Synthesis Series, 1976, p. 405-428.
18. **Lorenzo FR, Huff C, Myllymaki M, Olenchock B, Swierczek S, Tashi T, Gordeuk V, Wuren T, Ri-Li G, McClain DA, Khan TM, Koul PA, Guchhait P, Salama ME,**

Xing J, Semenza GL, Liberzon E, Wilson A, Simonson TS, Jorde LB, Kaelin WG, Jr., Koivunen P, and Prchal JT. A genetic mechanism for Tibetan high-altitude adaptation. *Nat Genet* 46: 951-956, 2014.

19. **Maltepe EF, S. J.** In: *Annual Review of Cell and Developmental Biology*, edited by Schekman R. Annual Reviews, 2015, p. 523-552.

20. **Martin DS, Goedhart P, Vercueil A, Ince C, Levett DZ, Grocott MP, and Caudwell Xtreme Everest Research G**. Changes in sublingual microcirculatory flow index and vessel density on ascent to altitude. *Exp Physiol* 95: 880-891, 2010.

21. **Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S, Angus DC, and Shapiro NI**. The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. *J Crit Care* 28: 913-917, 2013.

22. **Moore LG**. Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. *High Alt Med Biol* 4: 141-156, 2003.

23. **Moore LG, Charles SM, and Julian CG**. Humans at high altitude: hypoxia and fetal growth. *Respir Physiol Neurobiol* 178: 181-190, 2011.

24. **Niermeyer S, Andrade MM, Vargas E, and Moore LG**. Neonatal oxygenation, pulmonary hypertension, and evolutionary adaptation to high altitude (2013 Grover Conference series). *Pulm Circ* 5: 48-62, 2015.

25. **Niermeyer S, Andrade Mollinedo P, and Huicho L**. Child health and living at high altitude. *Arch Dis Child* 94: 806-811, 2009.

26. **Park AM, Sanders TA, and Maltepe E**. Hypoxia-inducible factor (HIF) and HIF-stabilizing agents in neonatal care. *Semin Fetal Neonatal Med* 15: 196-202, 2010.

27. **Pichler G, Binder C, Avian A, Beckenbach E, Schmolzer GM, and Urlesberger B**. Reference ranges for regional cerebral tissue oxygen saturation and fractional oxygen extraction in neonates during immediate transition after birth. *J Pediatr* 163: 1558-1563, 2013.

28. **Pisaneschi S, Strigini FA, Sanchez AM, Begliuomini S, Casarosa E, Ripoli A, Ghirri P, Boldrini A, Fink B, Genazzani AR, Coceani F, and Simoncini T**. Compensatory feto-placental upregulation of the nitric oxide system during fetal growth restriction. *PLoS One* 7: e45294, 2012.

29. **Pocivalnik M, Urlesberger B, Ziehenberger E, Binder C, Schwaberger B, Schmolzer GM, Avian A, and Pichler G**. Oropharyngeal suctioning in neonates immediately after delivery: influence on cerebral and peripheral tissue oxygenation. *Early Hum Dev* 91: 153-157, 2015.

30. **Pomeroy E, Wells JC, Stanojevic S, Miranda JJ, Moore LG, Cole TJ, and Stock JT**. Surname-inferred Andean ancestry is associated with child stature and limb lengths at high altitude in Peru, but not at sea level. *Am J Hum Biol* 27: 798-806, 2015.

31. **Postigo L, Heredia G, Illsley NP, Torricos T, Dolan C, Echalar L, Tellez W, Maldonado I, Brimacombe M, Balanza E, Vargas E, and Zamudio S**. Where the O2 goes to: preservation of human fetal oxygen delivery and consumption at high altitude. *J Physiol* 587: 693-708, 2009.

32. **Sakr Y, Dubois MJ, De Backer D, Creteur J, and Vincent JL**. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 32: 1825-1831, 2004.

33. **Song D, Li LS, Arsenault PR, Tan Q, Bigham AW, Heaton-Johnson KJ, Master SR, and Lee FS**. Defective Tibetan PHD2 binding to p23 links high altitude adaption to altered oxygen sensing. *J Biol Chem* 289: 14656-14665, 2014.
34. **Soria R, Julian CG, Vargas E, Moore LG, and Giussani DA**. Graduated effects of high-altitude hypoxia and highland ancestry on birth size. *Pediatr Res* 74: 633-638, 2013.
35. **Stroud MH, Gupta P, and Prodhan P**. Effect of altitude on cerebral oxygenation during pediatric interfacility transport. *Pediatr Emerg Care* 28: 329-332, 2012.
36. **Tissot van Patot MC, Ebensperger G, Gassmann M, and Llanos AJ**. The hypoxic placenta. *High Alt Med Biol* 13: 176-184, 2012.
37. **Tissot van Patot MC, and Gassmann M**. Hypoxia: adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2alpha. *High Alt Med Biol* 12: 157-167, 2011.
38. **Tissot van Patot MC, Murray AJ, Beckey V, Cindrova-Davies T, Johns J, Zwerdlinger L, Jauniaux E, Burton GJ, and Serkova NJ**. Human placental metabolic adaptation to chronic hypoxia, high altitude: hypoxic preconditioning. *Am J Physiol Regul Integr Comp Physiol* 298: R166-172, 2010.
39. **Top AP, Ince C, de Meij N, van Dijk M, and Tibboel D**. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. *Crit Care Med* 39: 8-13, 2011.
40. **Top AP, van Dijk M, van Velzen JE, Ince C, and Tibboel D**. Functional capillary density decreases after the first week of life in term neonates. *Neonatology* 99: 73-77, 2011.
41. **Urlesberger B, Brandner A, Pocivalnik M, Koestenberger M, Morris N, and Pichler G**. A left-to-right shunt via the ductus arteriosus is associated with increased regional cerebral oxygen saturation during neonatal transition. *Neonatology* 103: 259-263, 2013.
42. **van den Berg VJ, van Elteren HA, Buijs EA, Ince C, Tibboel D, Reiss IK, and de Jonge RC**. Reproducibility of microvascular vessel density analysis in Sidestream dark-field-derived images of healthy term newborns. *Microcirculation* 22: 37-43, 2015.
43. **van Elteren H, Reiss IK, and de Jonge RC**. Transcutaneous Microcirculatory Imaging in Preterm Neonates. *J Vis Exp* 2015.
44. **van Elteren HA, Ince C, Tibboel D, Reiss IK, and de Jonge RC**. Cutaneous microcirculation in preterm neonates: comparison between sidestream dark field (SDF) and incident dark field (IDF) imaging. *J Clin Monit Comput* 2015.
45. **Vargas M, Vargas E, Julian CG, Armaza JF, Rodriguez A, Tellez W, Niermeyer S, Wilson M, Parra E, Shriver M, and Moore LG**. Determinants of blood oxygenation during pregnancy in Andean and European residents of high altitude. *Am J Physiol Regul Integr Comp Physiol* 293: R1303-1312, 2007.
46. **Wehby GL, Castilla EE, and Lopez-Camelo J**. The impact of altitude on infant health in South America. *Econ Hum Biol* 8: 197-211, 2010.
47. **Yaron M, Niermeyer S, Lindgren KN, Honigman B, Strain JD, and Cairns CB**. Physiologic response to moderate altitude exposure among infants and young children. *High Alt Med Biol* 4: 53-59, 2003.

Acknowledgments

We wish to express our thanks to all mothers and their partners for their commitment to participate in this study. We are also indebted to the large community of pediatricians, obstetricians, nurses, laboratory experts and administrative personal of the Hospital EsSalud III in Puno (Peru) who all made this work possible especially Ximena Rodrigo Machicao, Sandra Y. Mamani Velarde, Yuliana Mamani Pilco, Paul Martines, and Jorge Anibal Osorio Terrones. We also thank Jorge Soliz and Silvia Urrutia from Quebec and La Paz, respectively, for their valuable help in classifying surnames as being of Andean or Hispanic origin, as well as Sir Peter Ratcliffe from Oxford and Jorge Soliz from Quebec for their valuable comments on the manuscript. We acknowledge the artwork and the professional English editing performed by Jeanne Peter (Zürich) and Ko Hagoort (Rotterdam), respectively. Finally, NNG and MG acknowledge the Swiss National Science Foundation, the Zurich Center for Integrative Human Physiology (ZIHP) and the Stiftung für wissenschaftliche Forschung an der Universität Zürich for their valuable financial support.

Author Contributions

IKMR and MG initiated this project. HAvE, TGG, LH, MR, IKMR and MG wrote the project outline and organized the equipment's transport to Puno. NNG, TGG and CRM performed the measurements in Puno with the help of SAM, PCP and APdC. TGG and HAvE did the measurements in Rotterdam. HAvE and NNG analyzed the data with the help of RCJdJ, DSM and LH. NNG, HAvE, MG and RCJdJ wrote the manuscript with help of IKMR, TGG, DSM, MR and LH.

Competing financial interest: The authors declare no competing financial interests.

508

509 **Table 1. Clinical parameters of the newborns at high and low altitude**

	High altitude: Puno (n=53)	**Low altitude: Rotterdam (n=33)**
Male gender (%, CI)	49.1 (27.1 – 51.0)	57.6 (40.8 – 72.8)
Caesarian section (%, CI)	35.9 (24.3 – 50.3)	60.6 (43.7 – 75.3)
Gestational age (weeks+days)[#]	39+0 (37+0 – 40+0)	39+5 (37+0 – 41+3)
Birth weight (grams) [#]	3310 (2590 – 4180)	3353 (2475 – 4450)
Rectal temperature (ºC) *	36.8 (0.3)	36.9 (0.3)

510 [#]Median (range)

511 * Mean (Standard deviation)

512

513

514 **Table 2. Crude and adjusted difference between Puno and Rotterdam for microcirculatory**
515 **parameters**

Variable	Difference between high altitude and sea-level (95% CI)			
	Unadjusted difference (95% CI)	p-value	Adjusted* difference (95% CI)	p-value
Total Vessel Density (mm/mm^2)	3.67 (2.68 – 4.66)	<0.001	3.57 (2.37 – 4.77)	<0.001
TVD small (mm/mm^2)	1.46 (1.02 – 1.91)	<0.001	1.14 (0.64 – 1.64)	<0.001
TVD medium (mm/mm^2)	2.79 (1.91 – 3.66)	<0.001	3.08 (2.00 – 4.16)	<0.001
TVD large (mm/mm^2)	-0.58 (-1.26 – 0.10)	0.129	-0.64 (-1.49 – 0.20)	0.132
MFI small (au)	-0.02 (-0.14 – 0.09)	0.688	-0.08 (-0.21 – 0.06)	0.261
MFI non-small (au)	0.03 (-0.04 – 0.09)	0.381	0.02 (-0.06 – 0.09)	0.646
HI small (au)	0.001 (-0.08 – 0.09)	0.854	0.02 (-0.08 – 0.13)	0.640
HI non-small (au)	0.03 (-0.04 – 0.09)	0.367	0.04 (-0.04 – 0.11)	0.368

516

517

Legends

Table 1: Clinical parameters of the newborns at high and low altitude

Clinical data from babies born at high altitude (Puno, n=53) and at sea level (Rotterdam, n=33) that were compared for TVD (see Fig. 2).

#median (range); * Mean (Standard deviation); CI = 95% Confidence Interval

Table 2: Crude and adjusted differences between microcirculatory parameters obtained from neonates born at high altitude and sea level.

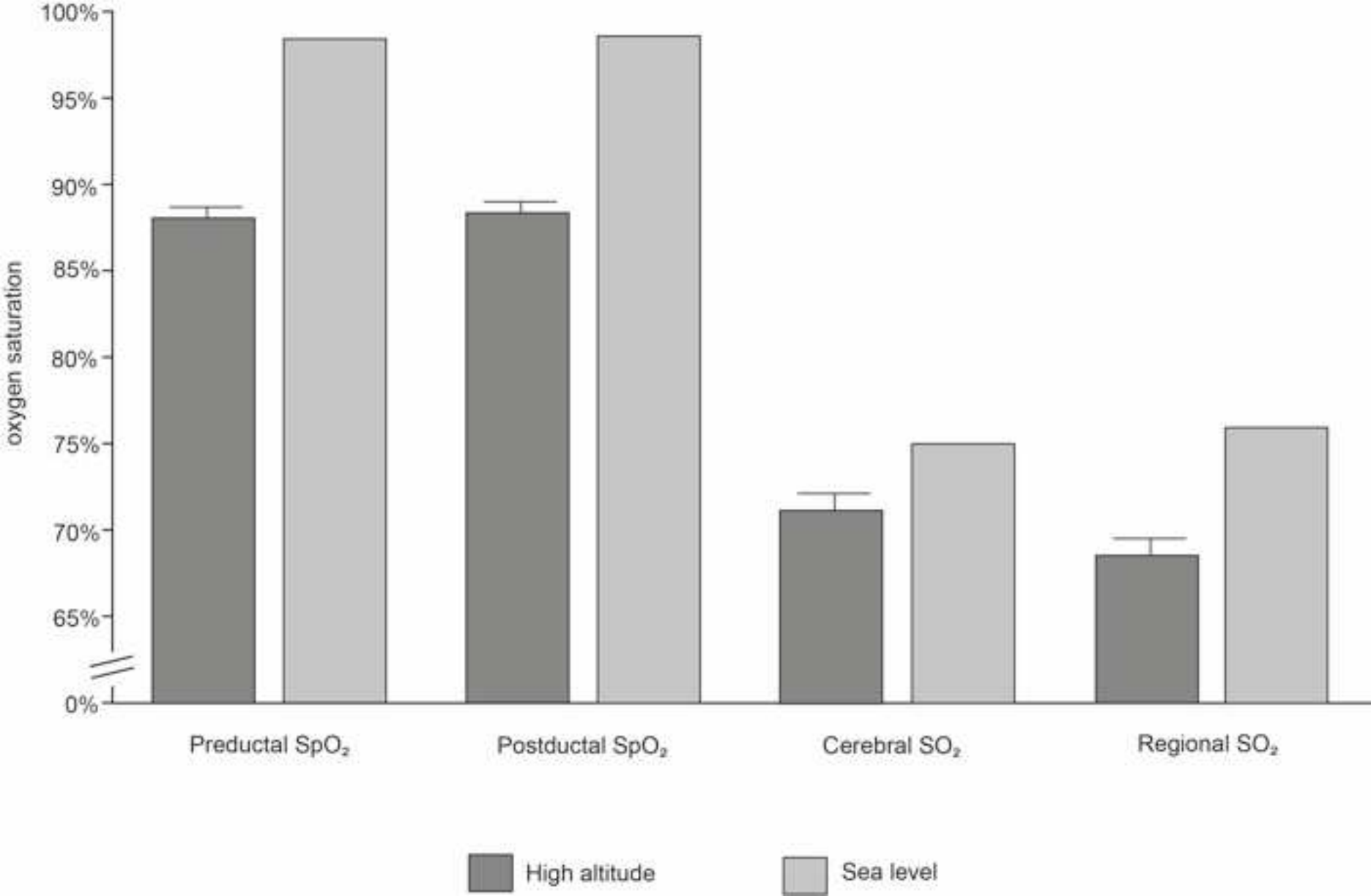
Crude data from the babies mentioned in Tab.1 (Puno n=52; Rotterdam n= 32) were adjusted (*) for country, sex, gestational age, birth weight z-score, Apgar score (5 minutes), mode of delivery, pregnancy (primigravida/multigravida) and rectal temperature as described in Materials and Methods. Small, medium and large vessels have Ø <10, 10-20 and 20 -100 μm, respectively. MFI: Microvascular Flow Index; HI: heterogeneity Index; CI= 95% confidence interval; au = arbitrary units

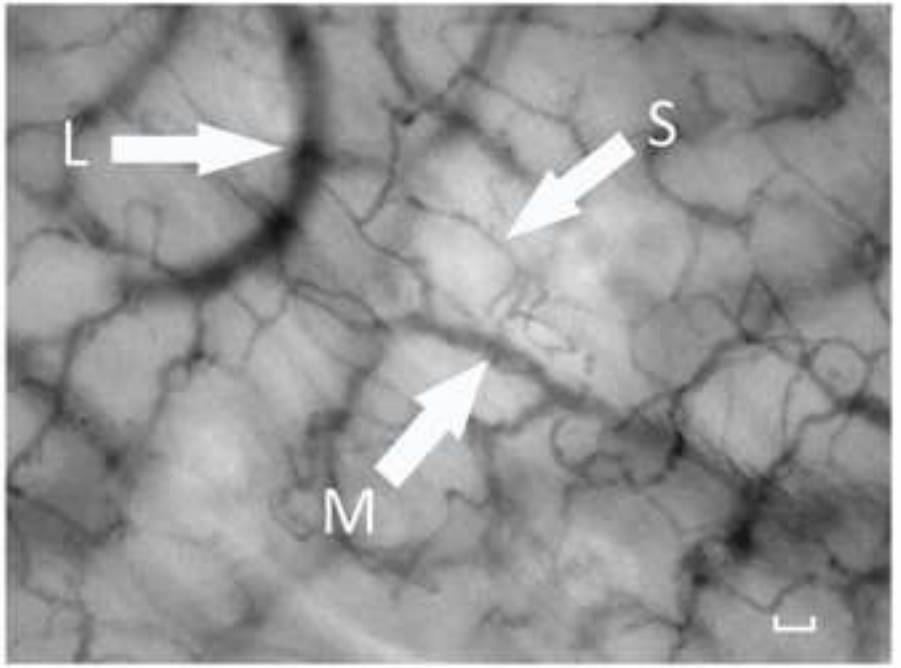
Fig. 1: Pre- and post-ductal arterial saturation (SpO_2) as well as cerebral and skeletal calf muscle (regional) oxygen saturation (SO_2) measured at high altitude are compared to sea level reference values.

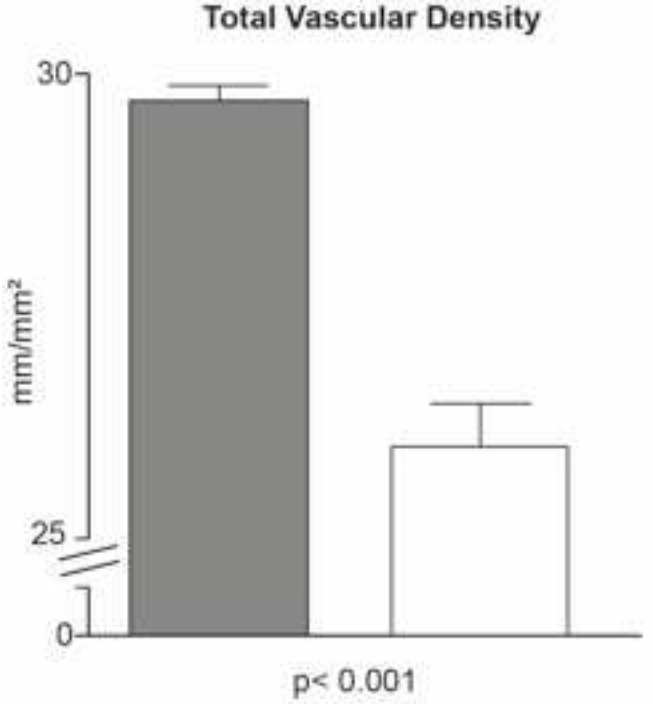
Pre- and post-ductal arterial saturation in newborns not older than 30 hours was measured as mentioned in Material and Methods. Skeletal muscle oxygen saturation was selected to mirror regional levels. The obtained data from high altitude babies (n=52) were compared to the published one at low altitude (0-326m, n=13,714 for pre- and postductal SpO_2 (13) as well as n=339 for cerebral (41). and n= 72 for regional SO_2 (29)). Error bars: SEM

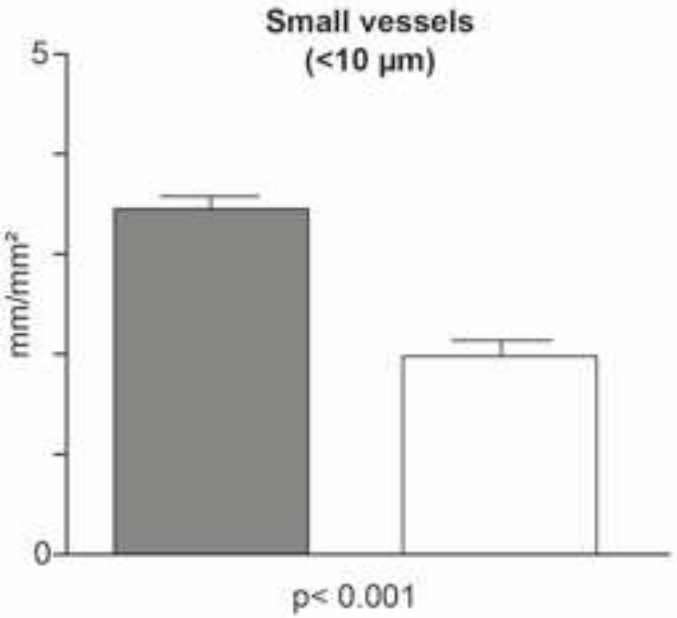
Fig. 2: Imaging and morphometric analysis of vessel density of the skin from newborns at high and low altitude.

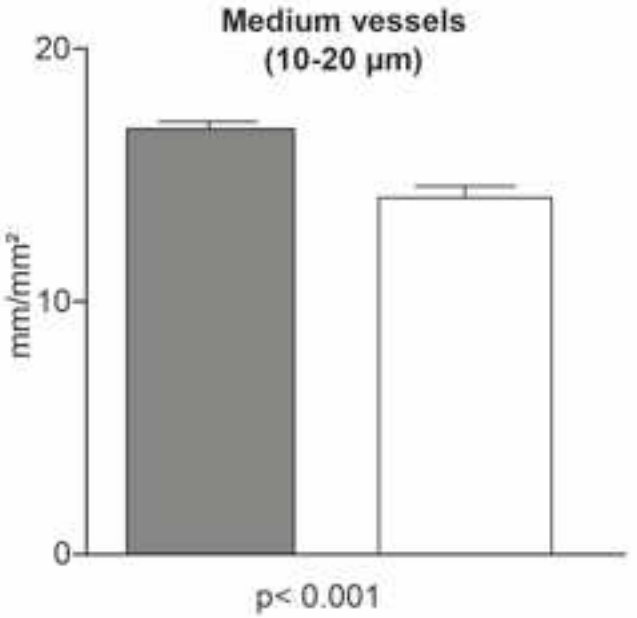
The image in the upper left shows a representative single shot of the video images obtained by a CYTOCAM. Small, Medium and Large vessels with Ø of <10, 10-20 and 20 -100 µm, respectively, are labelled. The bar represents 25µm.
The upper right graphic shows the unadjusted mean TVD measured in babies born at high altitude (dark bars, n=52) and at sea level (white bars, n=32). The lower graphs reflect the quantitation (unadjusted mean) of small, medium and large vessels.
Error bars: SEM

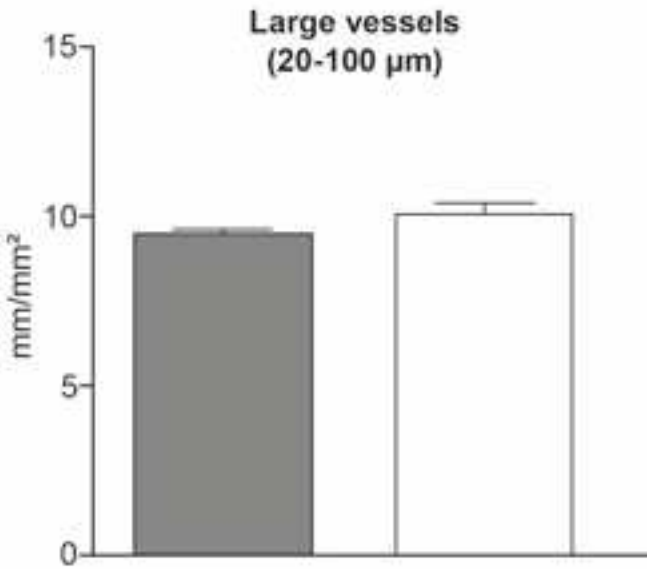

100%
95%
90%
85%
80%
75%
70%
65%
0%
oxygen saturation
Preductal SpO2
Postductal SpO2
Cerebral SO2
Regional SO2
High altitude
Sea level


L
S
M


Total Vascular Density
30
25
0
mm/mm²
p< 0.001


Small vessels
(<10 µm)
5
0
mm/mm²
p< 0.001


Medium vessels
(10-20 µm)
20
10
0
mm/mm²
p< 0.001


Large vessels
(20-100 µm)
15
10
5
0
mm/mm²


High altitude
Sea level